A next generation space antimicrobial: assessing microbial activity and reduction across the ISS

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Abstract

Microbial contamination in spacecraft poses a threat to crew health and operational integrity. Currently, microbial reduction aboard the International Space Station (ISS) relies on rigorous cleaning, which is time-consuming for astronauts. This study analyzes the ISS microbiome and applies a novel antimicrobial polymer coating to assess its effectiveness in mitigating microbial contamination. The polymer was coated onto five material types representing common objects interacted with by the crew. Each material had six coupons placed on six placards in various ISS locations, while five placards remained on Earth to accumulate microbes from passive and direct transfer. The trial lasted six months, utilizing microbiological cell cultures and genomic analysis to assess bacterial and fungal communities and identify species. We observed lower than expected microbial loads on ISS surfaces, indicating a reduced need for extensive cleaning. The polymer coating decreased culturable bacterial surface loads by 3.16 $\log_{10}$ compared to non-coated surfaces. Coated surfaces exhibited less genetic material and lower microbial species richness. Additionally, the polymer coating proved durable, persisting on ISS surfaces throughout the six-month period. This study demonstrates that the antimicrobial polymer coating effectively inactivates microbes and remains on surfaces in zero gravity environments. Its application is likely beneficial for preventing viral and bacterial contamination, with potential uses in long-duration spaceflight to enhance crew health and maintain spacecraft integrity. We also generated data suggesting an audit of current ISS cleaning practices could be warranted.

Introduction

The internal closed system of spaceflight environments provides a series of challenges in regards to microbial infection and microbe contamination (bacteria and fungi in particular) which can compromise materials, life-support systems and human health in what is an immunocompromising environment[1,2]. Microbiological risk factors for astronaut health include: food, drinking water, air, surfaces, payloads, animals, crewmembers, and personnel in close contact with the astronauts[3]. Potentially harmful microbial growth could also be exacerbated by mutation rate, replication and biomass of microbes in microgravity[4]. Although the effect of spaceflight on surface microbes has been minimally studied, it has been proposed that spaceflight does not dramatically alter the growth of surface microbes compared to Earth[5] with the caveats it does alter resistance to alkaline cleaning products[6] given environmentally sampled bacterial genomes from the ISS showed increased DNA repair activity, enhanced metabolism through mobile genetic elements, and furthermore evolution of potential pathogenic capabilities. Novel species have been identified in space[7], supporting the idea that the niche environment does promote growth of bacteria differently than on Earth[8–10] .

The importance and severity of notable spaceflight events relating to microbial growth in flights have been well summarized and include damage to equipment function[11], and several instances where uncontrolled microbial growth has threatened crew safety. For example, the Internal Active Thermal Control System (IATCS) on the ISS experienced uncontrolled planktonic bacterial and biofilm growth in the early years of the program[11]. This led to biofouling of various components and subsequent decreased flow rates, reduced heat transfer, initiation and acceleration of corrosion, and enhanced mineral scale formation.

Microbial spread can occur by three primary modes: aerosol, close contact, and fomite transmission by surface or object direct contact[12]. The risk of aerosolized particles can be somewhat mitigated by the engineering of air filtration and circulation systems[10]. Preventing fomite transmission, however,

currently relies on extensive cleaning at the expense of increased time in labor, extra payload mass/volume, cost, and indirect associated hazards[4]. Currently, cleaning aboard the ISS is performed weekly at a minimum[13]. Further, the cleaning methods vary in their efficacy, application effectiveness, pathogen deactivation time, and longevity, and may not be sufficient to inactivate or remove potential pathogenic or environmentally harmful organisms. In comparison, a persistent, fast acting antimicrobial that can be applied to surfaces would be useful in closed environments in space and as a public health measure on Earth in mitigating disease risks in a variety of settings (e.g., food industry, public transportation, etc).

Antimicrobial polymers applied to surfaces provide a useful means to control microbial growth including those of potentially pathogenic origin[14]. They have potential utility in preventing the formation of biofilms, relevant to hardware surfaces and regulated water systems aboard the ISS[13,15]. Previous evaluation of polymers to the ISS has assessed their hydrophobicity mode of action, and physical application method[16]. Here we discuss the anti-microbial impact of our novel surface treatment which provides a nanomechanical membrane rupturing mode of action, embedded microbe specificity and use as a persistent antimicrobial application in both spaceflight and terrestrial settings[14,17]. The coating consists of a polymer that has broad anti-bacterial and anti-viral activity, and most notably it allows for specific functionality to be built in to target multiple microbial strains at once[17]. The nanoparticles respond to touch, moisture, temperature and pH to activate microbe destruction. Further, it is fast acting, durable over a long-term period, water-resistant and has a degree of self-cleaning. While ground studies have shown the efficacy of this technology, testing this polymer on the ISS across two missions was aimed at understanding its function in the varied parameters of spaceflight including: microgravity, higher radiation levels, close quarter living and the material types used in spacecraft cabins. Building from an initial flight test in 2021[18], this study aims to assess the effectiveness of this antimicrobial polymer on microbes found in the ISS through bacterial quantification and identification via culture and complementary genetic methods, as well as durability on representative high touch surfaces[19].

Materials and Methods

The antimicrobial coating used here was initially flown to ISS on SpaceX-21 in December 2020 and returned to the ground on SpaceX-22 on July 2021[18]. The efficacy of the polymer coating was assessed for bacteriological and fungal growth. Microbial presence was less than expected which was at first thought likely due to the touching method not including a re-inoculation procedure and the delay in sample post-flight analysis given logistical challenges caused by the COVID-19 pandemic. While on this mission, post-landing pandemic staff limitations and site access impacted post-flight analysis, so data interpretations were limited but it did suggest relatively low contamination levels for ISS, at least in the assessed area with touch interactions as a variable.

The aim of the second flight, flown from November 2023 to April 2024, was to test the efficacy of the polymer coating against microbes in various locations on the spacecraft, gauge general microbial contamination levels and assess the durability of the polymer in prolonged spaceflight.

Experimental Hardware

There were 11 placards used in this experiment: six on the ISS and five grounded on Earth. Surface types were run under two possible seeding conditions: accumulations of fomite through physical touch interaction from crew (S1 Table 1), or from aerosolized particles. Controls were kept on the ground to provide a point of comparison. Experiments were run in parallel for the purposes of 1) assessing durability, and 2) microbial load assessed through culture and 3) genomic detection of deposition and taxon identification.

The sterilization of the material coupons and the preparation of the polymer mixture prior to coating can be found in the methods section of the supporting information 1. The polymer coating was adapted to be fluorescent to assess the durability of the coating on surfaces through time and exposure to touch. Five different surface type materials including seat belt strap, cargo bag fabric, medical gauze, tray table

material, and E-leather material were prepared onto sample coupons. The nanoworm dispersion was sprayed onto the surface at a distance of approximately 7 cm from the surface and dried completely at ambient temperature. The spraying-drying cycle was repeated four times. The coated and uncoated sample coupons were labelled and fixed to placards using Velcro in their designated locations. After assembly, three placards were packed into sterile zip lock bags sealed with Kapton® tape as negative controls. The orbital container with six sample placards was placed in a sterile zip lock bubble bag, sealed with Kapton® tape and sent to space.

On ground, the polymer coated sample coupons on the placard were separated into a control group and a treatment group. The control coupons remain undisturbed during the experimental period, whereas the treatment group was touched three times per week for a total of 24 weeks. On a fortnightly interval, each sample coupon was placed next to an uncoated coupon, irradiated via a 365 nm UV lamp at a distance of 10 cm from the light source, and the florescence captured via a camera.

Placard Placement & Sampling

Placards were placed in four locations on the ISS: three placards in the galley (Node 1), one placard in the exercise area (Node 3), one placard in the hygiene area (Permanent Multi-Purpose Module), and one placard in the toilet (Node 3) (S1 Fig. 1). Three placards were left on ground as comparisons. The material coupons on a placard were 2.5 cm in length and width with 0.5 cm between them (S1 Fig. 2). The locations chosen were viewed as the most likely areas to experience a larger presence of microbes when compared to other areas on the station[4].

The ISS fomite and durability placards were interacted with approximately every 48 hours as crew time permitted where the crew/personnel used a systematic touch regime to aid in the deposition of microbes and contaminants that may promote microbial growth, representing the fomite mode of microbe transmission. All samples on the fomite placards were touched (polymer coated and uncoated placards),

whereas the durability placard saw half the samples touched to assess potential polymer adhesion and/or removal through continual contact.

The remaining placards had no crew interaction throughout the six-month period, and instead relied on environmental deposition of microbes, representing airborne exposure to their respective locations listed above.

Placards intended for culture (S1 Table 1) were swabbed across their full surface on the ISS using Amies agar gel transport swabs to preserve viable microbes for transit to earth, with project team members connected through mission control at NASA's Marshall Space Flight Center to verify the crew's swabbing technique. All hardware was launched, operated, and returned at ambient temperature. Only the agar gel transport swabs were kept at 4 ^{o}C during return and handover to the payload developer at Kennedy Space Centre (KSC). On return of the swabs and shipment to the Boeing Huntsville laboratories, samples were assessed for live bacterial and fungal presence.

Microbial Culture Enumeration

Upon reception, swabs were vortexed in 3mL phosphate buffered saline. Ten-fold dilutions were performed on each sample from 10^{-1} to 10^{-2} in sterile phosphate buffered saline. Microbial enumerations were performed by plating 100 µL of these dilutions on 2 different media including a non-selective Tryptic Soy Agar (TSA) media for heterotrophic bacteria and a selective media for fungi (Sabouraud dextrose agar (SDA) with 0.025 g/L or 0.005% w/v chloramphenicol) per Standard Methods for the Examination of Water and Wastewater[20]. Media was selected to match current methods for monitoring microbial contamination on cabin surfaces on ISS[21].

Each sample dilution was performed in duplicate. TSA plates were incubated for heterotrophic bacteria for 7 days at 37 °C and colonies were counted at 2 days. Fungi plates were incubated at 25 ^{o}C room temperature for 5 days and counted colonies at 2 days and 5 days.

Microbial Isolate Identification

Identifications were performed by isolating each different colony morphology on a plate in pure culture. Bacterial identifications were performed to genus and species using fatty acid methyl ester gas chromatography and 16S sanger sequencing. Fungal isolates were identified using macroscopic and microscopic morphology. Pure bacterial cultures were identified firstly using fatty acid methyl ester (FAME) fingerprinting via the Microbial Identification System (MIDI, Inc., Newark, NJ). DNA was then extracted from microbial isolates using the Zymo Quick-DNA Fungal/Bacterial Mini-Prep Kit and amplified by using 27F/1492R full 16S universal primers for bacteria shown in S1 Table 2. Sanger sequences were trimmed for quality and assembled using BioEdit version 7.0.0. The bacterial consensus sequences were aligned against the SILVA SSU rRNA database using ARB software (http://www.arb-home.de/)[22] with a 95% minimum identify for genus-level and a 99% minimum identity for species-level identification. Bacterial isolates were also compared against the NCBI BLAST database using the same cutoff parameters. Organisms that were unable to be identified to species level but were identified to genus level were listed as “sp.” Organisms that were unable to be identified to genus level were listed as “Low match” or unidentified.

Placard Genetic Analysis

The aerosol-only hygiene placard was not sampled by space crew, and instead sampling was performed on Earth via qPCR or DNA sequencing to quantify bacterial presence regardless of survival. This involved the 'Hygiene' placard (S1 Table 1) which was not swabbed for culture, but instead sealed and returned to Australia’s University of Queensland for swabbing for subsequent qPCR or DNA sequencing, with methods described in the supporting information 2. For these tests each coupon was swabbed with a flocked swab (4N6FLOQSwabs™, Thermo Fisher Scientific, United States of America) wetted with 5 µL nuclease free H_20, swabbed over the full 2.5 x 2.5 cm^2 from top to bottom, left to right, and diagonally

174 bottom left to top right and then top left to bottom right per S1 Fig. 2. The interior and exterior of the
175 packaging were also swabbed in three different locations for 2.5 x 2.5 cm^2 as controls.

176 Genetic Sequencing

177 The DNA extraction process involved snapping the tip of each swab into a tube containing Solution CD1
178 and glass beads, followed by bead beating, heating, and centrifugation to obtain a lysate, which was then
179 further processed with the Qiagen DNeasy Powersoil Pro Kit (see S2). Quantitative PCR was performed
180 on the eluted DNA using specific primers to amplify bacterial and archaeal 16S rRNA genes, with the
181 resulting cycle threshold values analyzed via software. For amplicon sequencing, the 16S rRNA gene's
182 V3 and V4 regions were targeted using modified primers, and the library preparation followed Illumina's
183 workflow, including purification and indexing of PCR products before sequencing on the MiSeq system.
184 The analysis pipeline involved quality control of the sequence data, removal of primer sequences, and
185 processing with QIIME2 for feature selection and taxonomy assignment, ensuring high-quality reads
186 were utilized for accurate results (see S2). For a detail description of the DNA extraction, quantitative
187 PCR, Sequencing, and the genomic analysis pipeline please see the supporting information 2.

188 Genomic Analysis - Quantitation

189 One of the 30 total samples from the hygiene placards had extreme results, possibly due to contamination.
190 Since this sample appears to be from a different population for inference (contaminated vs
191 uncontaminated), each analysis was done with and without that sample. This analysis focused on the neat
192 dilution only, averaged over technical replicates for a single value per sample. There were more
193 “undetermined” technical replicates in the coated samples compared to the uncoated samples, which may
194 indicate some upwards bias for the results of the coated treatment.

195 Durability

196 We established that detection of an adjuvated fluorophore provided an accurate measure of polymer
197 presence[17]. The durability of the coating was thus analyzed using fluorescent light, where the intensity

of light emitted correlates with the quantity of the remaining surface coating. Prior to this, we assessed whether fluorophore exposure to UV light caused confounding 'quenching' that would compromise the use of this detection method to indicate polymer presence [data not shown, manuscript in progress]. Over 5 days, the polymer-fluorophore complex was applied to multiple surface types found in an aircraft cabin, and the level of fluorophore quenching was tested in an ambient context without human interaction, under multiple lighting conditions. There were no statistically significant changes between covered and exposed samples on each day for all lighting conditions and thus fluorescence of surface coating was deemed a valid metric for quantifying/assessing polymer presence. This experiment served the additional purpose of demonstrating the minimal invasiveness of both color and removal process. The durability of the surface coatings were assessed via ImageJ software.

Statistical Analysis

In this study, three distinct analytical approaches were used to assess how the material type and polymer coating impacted microbial activity on the sample coupons. The first examines the effect that coupon material type and polymer coating have on preventing microbial contamination and growth using microbiological cultures taken from the coupons. This analysis was restricted to the Galley – 3 placard coupons owing to insufficient growth/microbial presence on the other placards/coupons. Summary statistics were used to assess qualitative differences in $\log_{10}$ microbial colony forming units (CFU) per cm^2 between material type and the application of the polymer, displaying count information, the mean ($\bar{x}$) and standard deviation (σ) between each group. An analysis of variance was used to determine whether there was a difference in mean CFU between the materials and polymer coating. Owing to the low number of samples per material type, there was insufficient data to conduct a statistical test on the interaction between material type and the polymer coating, thus separate univariate analysis were used.

Due to the presence of overdispersion, a negative binomial generalized linear regression model with a log link was used to examine the polymer coatings antimicrobial activity in controlling microbial survival and growth. A constant (one $\log_{10}$) was added to bacterial counts to allow for counts to be expressed as a $\log_{10}$

223 increase and to adjust for zero count observations where samples had no growth. A dispersion parameter
224 (α) was included in the models to represent the ratio of the variance to mean $\log_{10}$ microbial CFU/cm^2.
225 Beta coefficients were exponentiated from a log scale, returning the outcome to a multiplicative $\log_{10}$
226 effect change on microbial CFU/cm^2. Model fit was checked using simulated residuals plots and no
227 problems were noted.

228 The second analysis examined the antimicrobial effect the coupon material type and polymer coating have
229 on the microbial species community and genetic material. This analysis was restricted to the hygiene
230 placard and samples taken from Earth ground controls. Summary statistics were used to identify and
231 quantify the relative abundance of genetic material by each detected taxon from genomic analysis
232 described above. Non-metric multi-dimensional scaling (NMDS) analysis[23] was used to characterize
233 species communities among the samples. An analysis of similarities (ANOSIM), a non-parametric
234 statistical test, was conducted to construct dissimilarity matrices to test whether there were statistically
235 significant differences between the composition of microbial species from coupon material types and
236 whether the coupon was coated or uncoated with the polymer. Multi-level pattern analysis was then used
237 to determine if the amount of genetic material present on the coupons significantly differed between
238 material type and between coated and uncoated coupons.

239 The third analysis examined how the number of microbial taxon and amount of genetic material varied by
240 material type and polymer coating. A linear model using the natural log of abundance as the response
241 variable and the polymer coating, material type, and their interaction as explanatory variables, was fitted
242 to the data. Model assumptions were checked using residual plots and assumptions of variance
243 heterogeneity and normality of errors were reasonably met. However, note that the model including the
244 unusual sample did not fit that point well, leading to that point as an outlier in the right-hand tail of the
245 distribution of residuals. Due to the log transformation, inference is to multiplicative differences in
246 medians. Discounting the outlier, a negative binomial generalized linear model with a log link was fit to

the number of species data, with the polymer coating, material type, and their interaction included as explanatory variables. Model fit was checked using simulated residuals plots and no problems were noted.

Statistical analysis was undertaken in R (Version 4.0.3, www.r.project.org)[24], using the latest compatible versions of packages 'MASS'[25], 'vegan'[26], 'ggplot2'[27], DHARMa[28], & 'indicspecies'[29] in RStudio (Version 2023.12.1)[30].

Results

There was minimal viable bacteria and fungi on all aerosol and fomite placards besides the aerosol Galley – 3 placard coupons, where 11 coupons showed viable bacteria, of which 19 of 40 of the swab samples (including duplicate replicates) had detectable viable bacteria (S1 Table 3).

Data used in the analysis to test the efficacy of the polymer coating and the composition of bacterial genera were a subset of the placards that includes the Galley – 3 placard and the duplicate culture samples for bacterial species only. The mean ($\bar{x}$) bacterial CFU/cm^2 across all coupons was 354 CFU/cm^2 with a standard deviation (σ) of 116.17 CFU/cm^2. Coated coupons had a lower mean bacterial CFU/cm^2 ($\bar{x}$ = 85.5, σ = 231.6) than that of the uncoated coupons ($\bar{x}$ = 622.5, σ = 950.1), with the data being overdispersed. This difference was found to be statistically significant (Fig. 1), where uncoated coupons had 3.16 $\log_{10}$ increase in bacterial CFU/cm^2 than that of coated coupons (β = 3.16, Confidence Interval (lower-upper) = 1.5-7.0, degrees of freedom (*df*) = 39, *p-value* = 0.003). Among coupon material types, there was no statistically significant difference in bacterial CFU/cm^2 (analysis of covariance, F = 1.26, df = 4, *p-value* = 0.306) (S1 Fig. 3).

Fig. 1. Negative binomial generalized linear regression coefficients (black point) and 95% confidence intervals (bars) of bacterial CFU/cm^2 for coated and uncoated materials.

There were eight bacterial genera found to make up five percent or more of the total abundance by CFU/cm^2 (Table 1). Three genera dominated the bacterial community abundance, *Neisseria sp, Streptococcus sp.* and an unidentifiable bacterium.

Table 1. Percentage (%) abundance of total identified CFUs and genetic material above 5% from bacterial cultures and genetic RT-PCR, respectively.

	Culture	**Genetic analysis**	
Unique Species	**ISS**	**ISS**	**Ground Samples**
Gemella haemolysans	8.05	-	-
Low match *Streptococcus sp.*	4.96	-	-
Neisseria sp.	28.42	-	-
Pseudomonas sp.	6.94	-	-
Rothia sp.	6.24	-	-
Staphylococcus sp.	7.18	6.7	26.9
Streptococcus sp.	17.36	-	8.8
Corynebacterium sp.	-	32.1	19.5
Cutibacterium sp.	-	2.7	44.8
Mitochondira sp.	-	11.5	-
Chloroplast sp.	-	47.0	-
Unidentified bacterium	19.87	-	-

* Identification of chloroplast sequences is included; however, it is important to note that since this analysis focused on 16S rRNA, the presence of chloroplasts and mitochondria is generally regarded from none microbial origins.

The microbial community and species found on the ISS coupons were distinctly different in composition to those found on the ground samples (S1 Fig. 4). From the genetic sequencing of the ISS samples, there

were no statistically significant differences found between the microbial communities on coupons coated or not coated with the polymer **(**ANOSIM R = -0.05, *p-value* = 0.962**),** nor among different material types **(**ANOSIM R = -0.02, *p-value* = 0.960**)**. Whilst the community of species didn't differ, there were statistically significant differences in the amount of genetic material for specific species. In the coupons not coated with the polymer, there were three species that were found to be strongly and statistically significantly associated with the uncoated coupons that included *Brevibacterium sp.* (*p-value* = 0.004), *Fusobacterium sp.* (*p-value* = 0.033), and *Anaerococcus sp.* (*p-value* = 0.042). Comparing the genetic sequencing of the ISS and ground control samples, there was a statistically significant difference found between the microbial communities from samples taken on the ISS verses those on the ground controls (ANOSIM R = 0.639, *p-value* > 0.001). Microbes that were found to be strongly, and statistically significantly associated with ISS samples are show in supporting information 1 Table 4.

From the genomic analysis on the coupons of the hygiene placard, we found that for all materials, with the exception of the gauze sponge and an outlier on the E-leather, the uncoated coupons had on average a greater abundance of genetic material than that of the coated coupons (Fig. 2.A).

Fig. 2. A. Observed abundance of bacterial 16S SSU rRNA gene copies by polymer coating and material type as measured by qPCR. The horizontal lines show the geometric mean of each group to mirror the results from the analysis with a log-transformed response variable. B. Observed richness values by treatment and material type plotted as points. The horizontal lines show the mean of each group. Each pane was allowed to have a different y axis scale. The outlier sample on the e-leather material is labeled with the sample ID.

The polymer coating and material type both had a statistically significant difference in microbial abundance (F = 6.4, df = 1,19, *p-value* = 0.020) and (F = 3.4, df = 4,19, *p-value* = 0.030) respectively, however; no statistically significant association was seen between the coating and material types (F = 2.0, df = 4,19, *p-value* = 0.143). The difference in material type was primarily driven by the tray table material

having the lowest median microbial abundance (Ratio median = 0.29, CI = 0.11-0.74, t = -2.7, df = 19, *p-value* = 0.013) (S1 Table 5).

Genetic cycle threshold (Ct) values were overall generally high with a low number of gene copies. A summary of the observed Ct results by polymer coating and material type for the neat dilution is shown in supporting information 1 Table 6. Species richness was, on average, seen to be greater in uncoated samples across material types, with the exception of E-leather and gauze sponge (Fig. 2B). Moreover, material type showed a significant difference in number of species even after controlling for the outlier sample in E-leather (Table 2).

Table 2. The type of material and its effect on species richness with and without the outlier sample from the E-leather material.

Effect	χ^2	*df*	*p-value*
Without outlier	27.3	4	< 0.0001
With outlier	43.7	4	< 0.0001

The polymer coating was found to remain on the surface of all material types regardless of whether they were touched throughout the experimental period or not, with the touched surfaces having a greater mean gray value than that of the controls on all material types (Fig. 3). Moreover, when taking bi-weekly measurements of the ground samples, there was no observable decline in the amount of polymer coating present on the surface of the touched surfaces compared with the control surfaces (Fig. 4).

Fig. 3. Remaining polymer from ISS surfaces by material type and exposure to touching.

Fig. 4. Longevity of polymer coating between touched and control surfaces throughout time on ground samples.

Discussion

Microorganisms are ubiquitous, being present in nearly all natural and built environments, including those in space. Microbes pose a challenge to space flight, as pathogenic microbes can impact crew health and environmental microbes can impact upon the spacecraft when they degrade vehicle materials or interfere with systems function. We explored microbial presence on different surfaces aboard the international space station (ISS) from different material types and tested the efficacy of a novel antimicrobial polymer. Here we examine a new previously described antimicrobial polymer[15,18] and its efficacy and durability in spacecraft in zero gravity aboard the ISS over a six-month period. The findings from our study provide valuable insights into the microbial community aboard the ISS and the efficacy of this novel antimicrobial polymer coating in mitigating microbial growth. We found that microbial growth across all sampling locations was limited, and where there were microbes present, our polymer coating was effective in reducing microbial load and growth.

Among the placards that underwent microbial culturing, the Galley - 3 placard exhibited sufficient growth across the coupons to determine a statistically significant reduction in microbial burden on the coated samples, achieving a 3 $\log_{10}$ reduction in bacterial colony forming units (CFU) per cm^2. This represents an approximate 99.9% reduction in bacterial presence on surfaces coated with the polymer, and is consistent with previous unpublished ground-based bacterial studies on the polymer coating. This reduction is noteworthy, especially when compared to the polymer's efficacy against viruses, such as influenza, which has shown an even greater efficacy[14]. The most growth observed on a single coupon was in the uncoated tray table material with 320 CFU/cm^2, which is, while higher, comparable to the 30 CFU/cm^2 reported in earlier studies[31]. This highlights that the polymer coating is still effective in reducing viable microbial growth when microbial presence is low.

We expected that the non-cultivation-based methods would detect a high abundance and diversity of microorganisms present on the coupons. However, the genetic analysis conducted on the hygiene placard

showed similar minimal growth seen in the microbiological cultures, confirming our initial finding of limited microbial buildup on the test coupon surface aboard the ISS. However, the cycle threshold (Ct) values from quantitative PCR (qPCR) were consistently high, which could imply bacterial deposition but not survival for most areas of the spacecraft. The overall amount of genetic material differed by coupon material type. This difference may be attributed to the texture, absorption, and physical properties of the material where microbes may penetrate deeper into porous surfaces or have available metabolites to promote growth., Notably, the seat belt material had statistically significantly greater genetic material abundance than compared to the tray table material. However, we found no statistically significant difference in bacterial CFU/cm^2 by material type. We originally expected the hygiene placard to show greater aerosolized particles due to exfoliation behaviours[32], yet this was not observed, perhaps this could be attributed to the higher air filtration and flow rates on the spacecraft, an paradigm analogous to aircraft[10].

The species richness observed in our samples was intriguing with an outlier from one sample having both high levels of DNA and species richness. This suggests that the microbial community is not merely a result of a single CFU expanding but rather represents a diverse community of species. The polymer's durability further showed minimal degradation of the coating over the six-month study period. However, if the polymer is applied in conjunction with cleaning, an adhesion promoter may be required to counteract its potential removal but we note part of the goal of the polymer’s presence is to avoid cleaning. Due to the low bacterial counts and genetic read counts from the aerosol placards, we cannot make definitive comments on the correlation between microbial load and durability. Future studies could explore this relationship further, as while we feel it is unlikely, higher microbial loads could inundate the polymer and lower the polymer's efficacy.

The lack of culturable organisms on the fomite placard raises questions about the potential for self-cleaning during surface touch events. One possibility is that the microbes deposited via touch were subsequently ingested, spread around the ISS environment, or potentially concentrated at specific areas

where we did not have placards. Our results indicated that there was a greater microbial diversity in ground samples compared to those from the ISS, suggesting a limited source of microbes in the space environment. The positioning of the placards on the ISS may have influenced the microbial communities observed, with potential sources including foodborne and fecal bacteria, and microbes from the human microbiome. The bacteria identified were generally not pathogenic; however, they may still pose potential concerns for astronauts on long-term missions, where immunosuppression could exacerbate risks. Notably, two of the three uncultured bacteria more significantly associated with the uncoated communicated were gram-positive, with a mix of aerobic and anaerobic species. The presence of the *Neisseria* genus, which includes pathogenic species such as gonorrhea, is particularly noteworthy, as many species within this genus are primarily soil-dwelling and can be infectious.

Our findings align with previous studies that have reported higher levels of *Staphylococcus* and *Streptococcus* in space compared to ground samples[33]. However, the higher diversity observed on Earth suggests that the genera with the greatest abundance in space may be more diluted as a percentage rather than indicating a higher overall presence in the ISS. The presence of *Penicillium*, which was tied for the highest abundance of yeast with *Rhodotorula* in our analysis, supports findings from other studies as leading contributors to the microbial community abundance[34]. Moreover, our results indicate that the microbial community of the ISS differ significantly from those found on Earth. While in Venkateswaran et al. (2014), more than 90% of all genetic sequences were assigned to four bacterial genera (*Corynebacterium, Propionibacterium, Staphylococcus,* and *Streptococcus*), in our study, these genera comprised only 24% of the data (9.6%, 0.05%, 10.7%, and 3.6%, respectively)[35]. This difference highlights the unique microbial ecology of the ISS, its potential to change through time in a closed-loop system, and for microbial adaptation in space.

The source of microbes aboard the ISS remains a topic of interest for crew health, environmental systems, food production, and vehicle safety. Previous studies have suggested that the microbial communities on the ISS are more similar to those found in human homes rather than to those on the human body[36]. Our

399 findings support this, as we identified environmental bacteria such as *Neisseria* and *Staphylococcus*,
400 which are likely to originate from clothing fibers, skin, food, and other sources[32]. In the absence of
401 gravitational settling of microbes onto surfaces, the ISS may allow larger particles to persist in the
402 internal atmosphere in areas where air flow doesn't readily move particles to the air filtration system,
403 creating unique exposure environments for astronauts. The lack of microbial abundance found here may
404 have future impacts on research and operational protocols aboard the ISS. For instance, the lack of
405 microbes could reflect the cleaning efficacy by the crew and ventilation system, and the potential of
406 overcleaning, where there may be a need to balance maintaining a sterile environment and the
407 practicalities of cleaning protocols, reducing the valuable time crew required for cleaning. Despite the low
408 bacterial counts found, the closed environment of the ISS may present niche microbial challenges where
409 hotspots for microbial accumulation may exist via touch or aerosol deposition and were simply not
410 incorporated in this study. As an example, the positioning of placards, such as Galley – 3, which was
411 located above other galley placards, could verify airflow dependent microbial deposition. Future
412 modeling simulations could aid in predicting the optimal placement for antimicrobial interventions,
413 particularly in high-traffic areas.

414 A strength of the polymer used in this study is that there is no associated toxicity to its use and to
415 humans[17]. However, one limitation of our study is the potential selection for highly adapted microbes
416 aboard the ISS, such as spore formers, which our polymer may not effectively target[5]. Additionally, the
417 low biomass challenge we faced, coupled with the different methods used in our culture laboratory, may
418 have influenced our results[37]. The tightly controlled cabin humidity aboard the ISS may have
419 contributed to the low bacterial presence observed on the surface coupons. With low moisture levels,
420 there is limited moisture deposition on the coupons, which further inhibits active microbial growth and
421 proliferation. The logistics of space related payloads impacted our approach and sampling procedures,
422 including the number of samples and replicates taken, as well as the timing of sample collection[5].
423 Previous efforts to detect microbes in space have shown that while microbiological cultures assist in

revealing post-flight microbial viability, inherent issues with microbial die-off during sample return have caused genomic analysis to have a mainstay-place in analysis and assessment of microbial levels. Here we applied microbiological culturing and genomic analysis to capture both viable and nonviable organisms, and have findings representative of what microbes circulate in the ISS.

Our study highlights the low microbial counts observed from natural environmental deposition and from the touching interaction from the astronaut crew, with deposited bacteria not actively replicating at high levels. We show an approximate 99.9% reduction in bacterial presence on surfaces with the use of the polymer coating. The durability and presence of the polymer persisted the entire duration of the six-month experimental period, indicating its potential for long-term use in space environments. Future work should focus on evaluating hotspots based on airflow, crew contact, and swabbing, followed by assessing this polymer coating as an intervention in these targeted areas. Additionally, understanding the role of touch in the ISS context may provide insights into microbial removal and cleaning efficacy. This may further our understanding how we may optimize cleaning regimes aboard crewed space vehicles. Overall, our findings contribute to the ongoing efforts to ensure astronaut health and safety in the unique environment of space.

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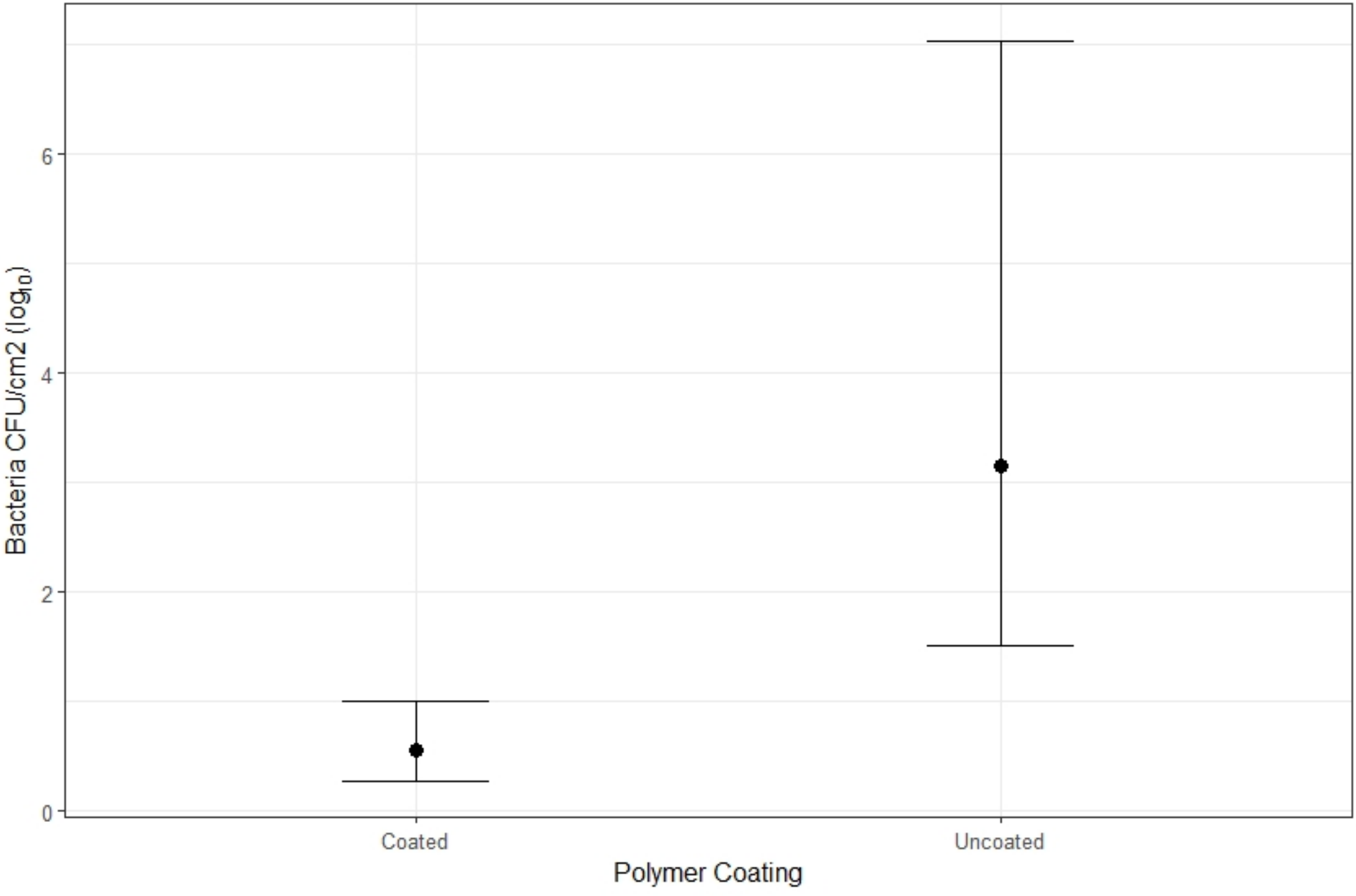
Bacteria CFU/cm2 (log10)
0
2
4
6
Coated
Uncoated
Polymer Coating

Figure 1

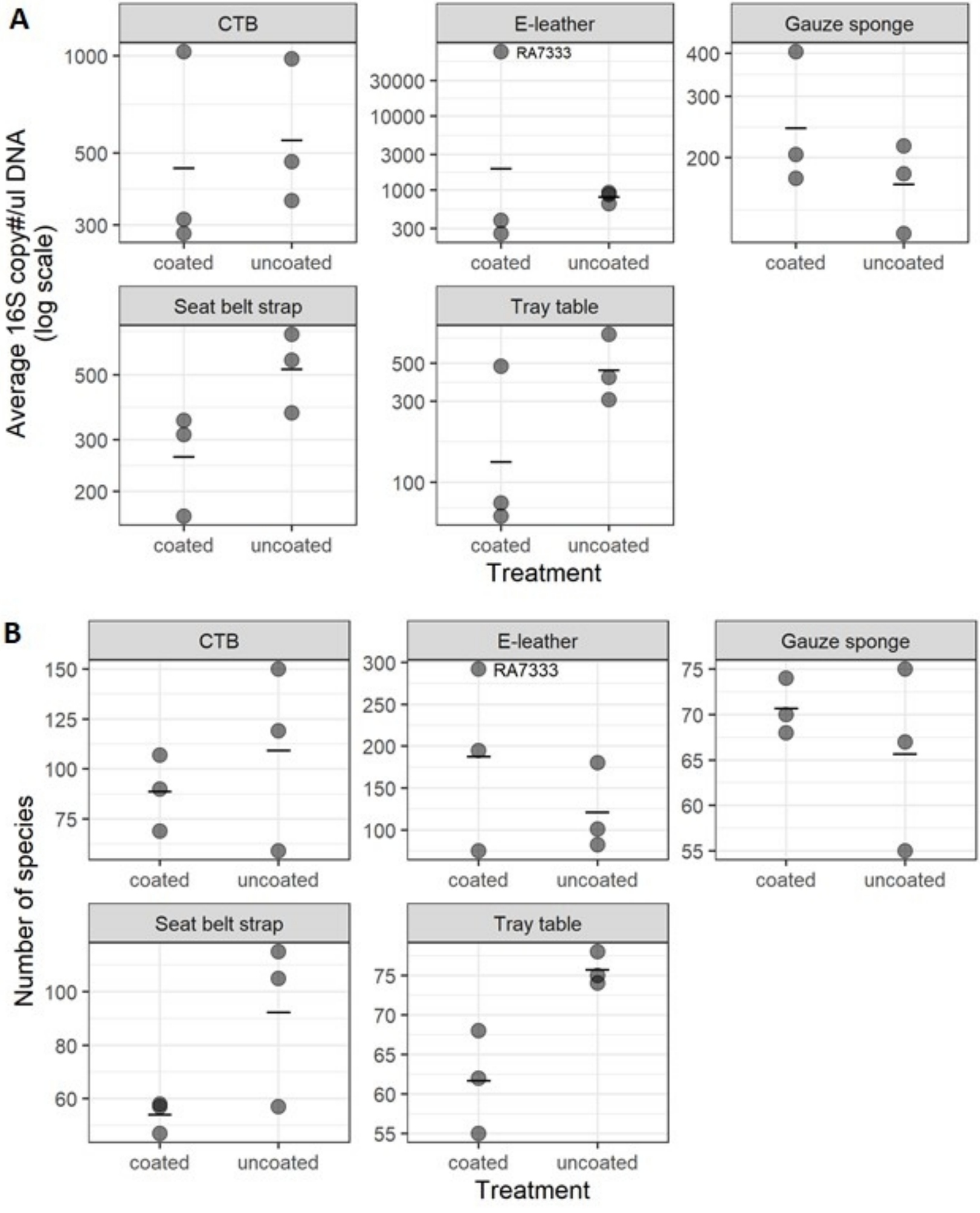
A
CTB
E-leather
Gauze sponge
Seat belt strap
Tray table
RA7333
Average 16S copy#/ul DNA (log scale)
1000
500
300
30000
10000
3000
1000
300
400
300
200
500
300
200
500
300
100
coated
uncoated
Treatment
B
CTB
E-leather
Gauze sponge
Seat belt strap
Tray table
RA7333
Number of species
150
125
100
75
300
250
200
150
100
75
70
65
60
55
100
80
60
75
70
65
60
55
coated
uncoated
Treatment

Figure 2

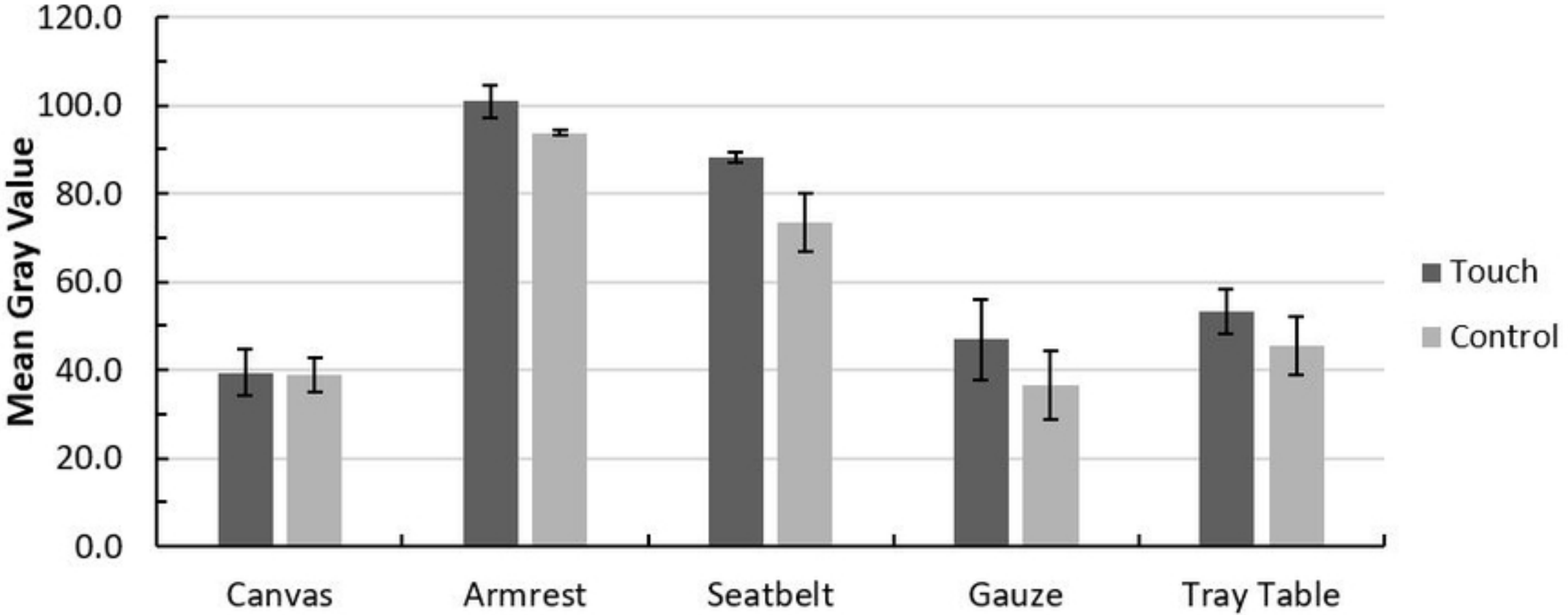
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Mean Gray Value
Canvas
Armrest
Seatbelt
Gauze
Tray Table
Touch
Control

Figure 3

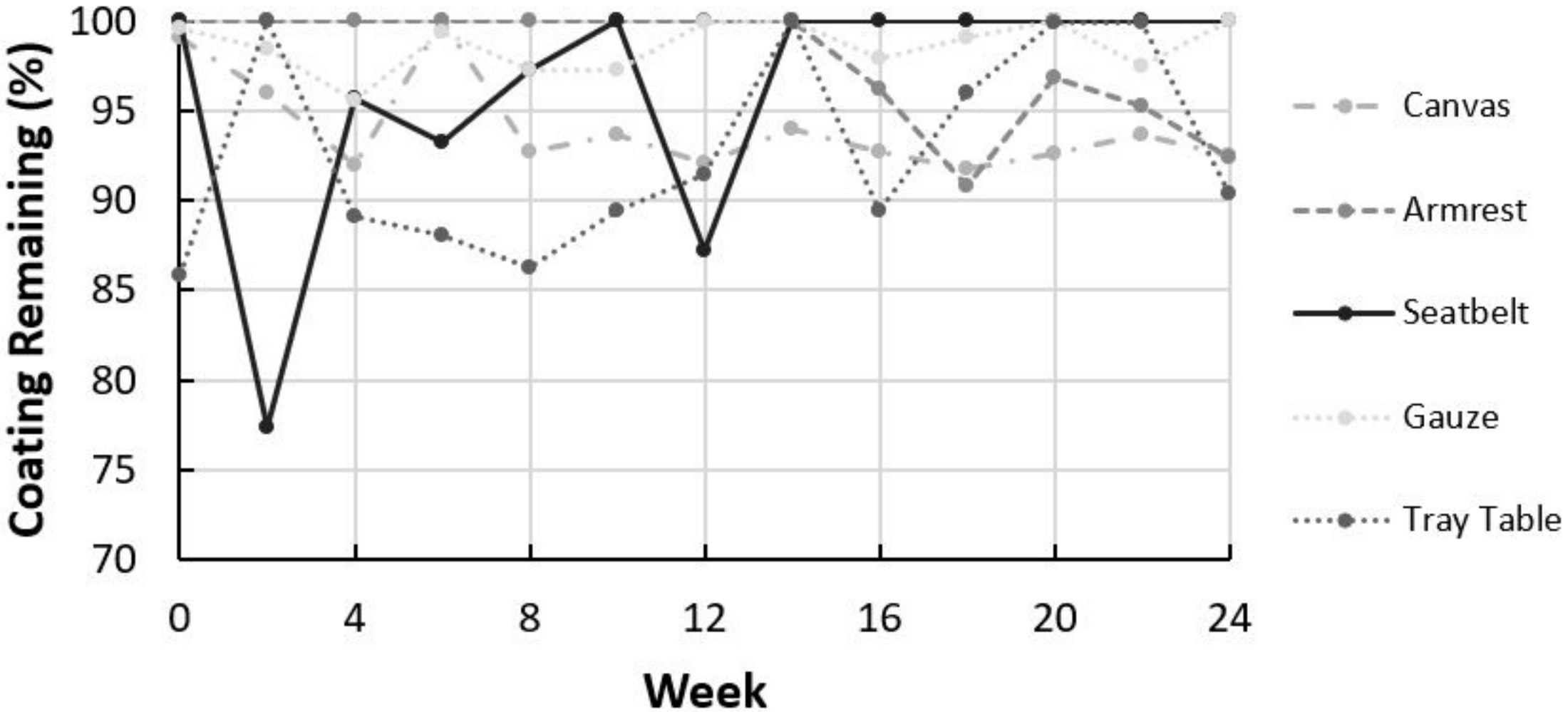
Coating Remaining (%)
100
95
90
85
80
75
70
0
4
8
12
16
20
24
Week
Canvas
Armrest
Seatbelt
Gauze
Tray Table

Figure 4